The Huxley Lecture

ON

RECENT STUDIES OF IMMUNITY,

WITH

SPECIAL REFERENCE TO THEIR BEARING ON PATHOLOGY.

Delivered at the Opening of the Winter Session of Charing Cross Hospital Medical School on October 1st, 1902.

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Gentlemen,—You will readily believe that with my deep appreciation of the high honour conferred by the invitation to deliver the fourth Huxley Lecture there was joined a sense of great embarrassment in being called upon to follow in this office three such leaders of world-wide fame as Sir Michael Foster, Professor Virchow, and Lord Lister. But the letter of the Committee of the Charing Cross Hospital Medical School stated that the choice of a successor to these great names was "a tribute of our admiration for the great army of scientific workers on the other side of the Atlantic." While I cannot assume to occupy any other place in this army than that of a soldier in the ranks, I felt that if my acceptance of this invitation could be regarded as in any sense an expression of appreciation by American workers in science of the commendation and good will of our British colleagues, of our large indebtedness to them, of our sense of the common interests, the comradeship and the kinship of the English-speaking peoples on both sides of the ocean, I should not decline, even if summoned to occupy a position of danger.

There was another consideration which I may be permitted here to mention. Through Huxley there is, if not a bond, at least a link between the Charing Cross Hospital Medical School and the Johns Hopkins University. This lectureship was founded to commemorate the fact that Huxley received his entire medical education at the Charing Cross Hospital Medical School. While throughout America the name of Huxley is held in high honour as that of a great discoverer and interpreter in science, and while the influence which he has exerted upon popular as well as scientific opinion through those messages peculiarly fitted to the needs of English thought is not less there than among his own countrymen, we at the Johns Hopkins University have special reasons to acknowledge our gratitude to him. He crossed the ocean to deliver the principal address at the opening of this University in 1876, and he then gave utterance to ideas concerning university, and especially medical, education which were at the time and have remained an inspiration and a guide to us. Then, too, the Johns Hopkins University owes to Huxley and to Michael Foster the accession to its faculty of my lamented colleague, Newell Martin, who by the introduction and development of the biological methods and conceptions of his teachers gave such new directions and so great an impulse to biological study in America that his own work and that of his pupils started for us a new era in this department.

The first Huxley lecturer has made it unnecessary for his successors to dwell upon Huxley's studentship at the Charing Cross Hospital, upon the important influence which this had upon his career, or upon his great services to medical science, although his chief title to fame lies outside of the domain of medicine. I should like, however, to quote a passage, although it must be familiar to you, from Mr. Leonard Huxley's charming *Life and Letters* of his father, which has appeared since the date of Sir Michael Foster's lecture, for it shows that "it was at Charing Cross Hospital where Huxley first felt the influence of daily intercourse with a really able teacher." He says:

> No doubt it was very largely my own fault, but the only instruction from which I obtained the proper effect of education was that which I received from Mr. Wharton Jones, who was the lecturer on physiology at the Charing Cross School of Medicine. The extent and precision of his knowledge impressed me greatly, and the severe exactness of his method of lecturing was quite to my taste. I do not know that I have ever felt so much respect for anybody as a teacher before or since.

Wharton Jones, who will doubtless be longest remembered as the discoverer of the amoeboid movements of the white blood corpuscles, was an experimental physiologist and pathologist of much originality, and it seems to me that there has not been, even in his own country, so full a recognition of his work as its importance merits.

Before passing to the special theme of this lecture it is fitting that I should pause, if only for a moment, to call to mind with affection and reverence that recently departed great man who honoured and delighted you four years ago, and who has conferred such high distinction upon the office of Huxley lecturer. When one considers the full import of the discovery and establishment by Virchow of the principles of cellular pathology, that this constitutes the secure foundation upon which nearly two generations have built and future generations will continue to build the edifice of scientific medicine, I do not know what greater name there is in the whole history of medicine than that of Rudolf Virchow. How noble his character! With what amazing industry, versatility, and keenness of intellect did he fruitfully cultivate the new fields which he had opened to research as well as other departments of science! With what devotion and beneficial results did he give his time and abundant knowledge to the service of the public and of our profession! We mourn the loss of a hero of medicine and of science, a benefactor of his race, and we rejoice in the rich fruitage of a long and well-spent life.

The first place in experimental medicine to-day is occupied by the problems of immunity and, in accordance with the trust of the Huxley lectureship, which provides that the lecture shall relate to "recent advances in science, and their bearing upon medicine and surgery," I have chosen for my theme Recent Studies of Immunity, with Special Reference to their Bearing on Pathology." As it would be hopeless to attempt a complete review of this broad subject within the space of a single lecture, I shall dwell more particularly upon certain of its aspects, not always of necessity the most important ones, which I conceive to be less familiar to most physicians, or which have engaged my attention, although much which I shall say is of course known to those who have followed the results of recent work in these new lines of investigation.

Under "studies of immunity" I have included, as a matter of convenience, though not with strict accuracy, investigations which, although the direct outgrowth of those primarily directed toward a solution of the problems of immunity, have extended far beyond these bounds, and have revealed specific properties of cells and fluids in health and disease of the broadest biological interest. We find illustrated here the familiar fact, nowhere more important to recognize than in medicine, that the sciences are interdependent, that discovery in one field sheds light in most diverse and often unexpected directions, and opens new paths to research. We shall see also exemplified the fructifying influence upon the advancement of knowledge of the discovery and application of new methods of investigation.

In endeavouring to follow in its intimate workings the contest of the living body with its invaders, the attention of investigators has naturally been drawn both to the action of the cells and to the properties of the fluids of the body in this struggle, to the latter sometimes without sufficient consideration of the dependence of the humours in their composition upon the cells. Each of these lines of study, whether followed separately or conjointly, has led to the discovery of important facts relating to the mechanism of immunity.

We owe to Metchnikoff and his pupils the most important observations concerning the direct participation of leucocytes and other cells in the processes of infection and the production of immunity. Whatever attitude one may take toward Metchnikoff's well-known phagocytic theory of immunity, one must recognize the wealth of new facts which he has brought to light, and must admire the skill and fertility of resource with which for two decades he has defended this theory against severe assaults, and he has done so, in my judgement, with a large measure of success. With wonderful ingenuity in his recent book on immunity he rescues the phagocytes and applies to a deeper insight into their activities results of his opponents' work.

The other line of research, in some respects more important, was opened by Nuttall in 1888, working in Flügge's laboratory, by his systematic study of the antibacterial properties of the body fluids, particularly of the blood serum. It is true that there were previous indications of the power of fresh blood to kill bacteria; indeed, if one wishes to trace this matter historically to its roots he must go back to John Hunter, who was quite familiar with the antiputrefactive power of fresh blood, although of course he knew nothing of bacteria. Hunter showed that putrefying fluid could be added in small quantity to fresh blood without setting up putrefaction; and in elaborating his favourite doctrine of the "living principle of the blood" he interested himself greatly in certain phenomena which, interpreted in the light of our present knowledge, are clear anticipations of some recent findings.

After Nuttall our knowledge of the bactericidal power of the blood serum was extended by Buchner and others; but the next advance of fundamental importance in this direction was Pfeiffer's discovery in 1894 of the quick extracellular disintegration and solution of cholera spirilla in the peritoneal cavity of immunized guinea-pigs or in that of normal guinea-pigs treated with immune serum, and of the presence in the immune serum of a specific substance concerned in the bacteriolytic process, although by itself without bactericidal power.

In the meantime Behring had made his great discovery of antitoxic immunity and of the protective and curative value of antitoxic serum, and Ehrlich had done much to elucidate the nature of this form of immunity. It soon became apparent, however, that immunity from the great majority of bacterial infections does not depend in the main upon the antitoxic principle. The attention of bacteriologists, therefore, was drawn more and more to the so-called "Pfeiffer phenomenon," which was found to be of great general significance; and starting from this, and especially from the investigation of the analogous and much more readily studied solution of red corpuscles by foreign serum, there has followed in rapid succession up to the present time a series of new and most interesting discoveries and conceptions with which are connected many names, but most prominently those of Metchnikoff and Bordet, and of Ehrlich and Morgenroth.

Through these various studies of immunity we have become acquainted with an important physiological capacity of the healthy organism, the extent, and in most instances the existence, of which was unsuspected until quite recent years. This capacity is the power to produce substances specifically antagonistic to all sorts of foreign cells and cellular products and derivatives. The substances capable of inducing this immunizing reaction appear to be mainly of an assimilable, albuminous nature, or at least intimately associated with such material, although it has been proved that certain non-albuminous derivatives of proteids have the same power.[1] The mode of antagonism of the specific bodies formed in response to the reception within the living organism of substances capable of inducing the necessary reaction varies with the nature of these latter substances, and consists in such diverse manifestations as neutralization of poisons and of ferments, injury or destruction of cells, associated with characteristic morphological changes, cessation of motility of cells or their appendages, agglutination of cells, precipitation, and coagulation. In accordance with these different effects, the corresponding antagonistic bodies, or antibodies as they are called, are classified as antitoxins, antienzymes, cytotoxins, agglutinins, precipitins, and coagulins, and even against these bodies, with the exception of the antitoxins, antagonists have in turn been produced. All of these bodies are in varying, but usually high, degree specific with reference both to the nature and to the source of the material upon which they exert their characteristic effects.

The cytotoxins or cytolysins include not only the bacteriolysins and haemolysins but also a great number of other cellular toxins present in the serums of animals which have received injections of cells from a different species. To every cellular group of an animal species there appears to correspond a specific cytotoxin. To designate these various cytotoxins such self-explanatory names as leukotoxin, spermotoxin, nephrotoxin, neurotoxin, thyreotoxin, syncytiotoxin are used. Their specificity extends not only to the nature of the cells but also to the species of animals furnishing the cells used for their production.

One of the most important results of recent work is the separation of these specific antibodies into two groups, in one of which, represented by the antitoxins, the antagonists are single bodies; while in the other, represented by the cytolysins, the antagonistic effect requires the co-operation of two bodies. Of these two bodies the one which actually destroys the foreign cells, or induces other specific effect, is normally present in the cells or fluids of the organism, but it seems incapable of action without the intermediation of a body which is distinguished from it by greater resistance to heat, and which is produced by the immunizing reaction, although it may also be normally present in smaller amount.[2] The two elements composing a cytolysin exist quite independently of each other, so that one may be present without the other, or be artificially removed without affecting the other. Of the multitude of names proposed for these cytolytic components those most commonly used for the body which is the specific product of immunization, although it may also exist normally, are intermediary body, immune body, amboceptor, sensitizer, fixative, preparative, desmon, and for the other body complement, alexin, cytase. It is this latter body which contains the atomic group described as toxophoric or zymophoric.

Concerning the source, mode of action, and constitution of the specific antagonistic bodies we are very imperfectly informed. That they are of cellular origin seems certain, and Ehrlich with great ingenuity, on the basis of a brilliant series of experiments, has advanced a hypothesis regarding them which, in my opinion, better than any other hitherto suggested accords with the known facts, and in promoting discovery has already done the greatest service of which a working hypothesis is capable. Ehrlich has so recently and so fully in the Croonian Lecture presented before English readers his hypothesis of the side chains or receptors and the basis for it, that I need only recall to your minds his conception that the toxins, cells, and other substances which lead within the living body to the production of antitoxins, cytolysins, and other antagonists have this capacity only through the possession of specific affinities, called haptophore groups, for corresponding haptophore groups belonging to side chains or receptors of certain cellular constituents of the body, and that, in consequence of this appropriation of receptors, others of like nature are reproduced in excess of the needs of the cell, and these being shed into the lymph and blood there constitute the antitoxins, intermediary bodies, agglutinins, and other specific antagonists. The antitoxic receptor has only a single combining affinity, which is for the toxin, whereas the cast-off receptors constituting the intermediary bodies of cytolysins have at least two affinities (hence called amboceptors by Ehrlich), one of a more highly specialized nature being for the invading bacteria or other foreign cells, and the other for the complement.[3] The antibody enters quantitatively into definite chemical union with its affinitive substance. The essence of Ehrlich's theory concerning antitoxin is thus tersely expressed by Behring: "The same sub-

[1] Specific precipitins have been produced by injection of crystalline and other so-called pure proteids. Obermayer and Pick produced immune bodies by the use of non-albuminous products of tryptic digestion of certain albumins. Jacoby has shown that the specific body concerned in ricin immunization is non-albuminous. Klein obtained entirely negative results with injections of starch, glycogen, glucose, gum arabic, and gelatine.

[2] Metchnikoff believes that the complement or cytase, which in his opinion exists under normal conditions solely within cells, not free in the plasma, acts in natural immunity without the co-operation of an intermediary body or fixative, the latter being concerned only in acquired or artificial immunity. The evidence seems, however, to favour the view that in this regard the conditions are similar in both forms of immunity, the main difference being the presence of a much larger amount of the specific immune body in the latter.

[3] According to Ehrlich's latest conception, resulting from investigations to demonstrate the multiplicity of complements, an amboceptor has a single cytophilic affinity, and a number of complementophilic affinities differing in their avidity for various complements. He regards the agglutinins, precipitins, and coagulins as uniceptors of more complex structure than the antitoxins, but Bail has recently brought evidence to show that agglutinins, like cytolysins, are composed of two elements. For the purposes of this lecture, it is not deemed necessary to enter into these or many other details of this complicated subject. For comprehensive and admirable critical reviews of recent theories of immunity and Ehrlich's hypothesis of the receptors, I would refer to Professor Ritchie's papers in the *Journal of Hygiene*, vol. ii, No. 2, and succeeding numbers; and to Dr. Aschoff's paper in *Zeitschrift f. allgem. Physiologie*, Bd. iii, Heft 3.

stance which, when incorporated in the cells of the living body, is the prerequisite and condition for an intoxication becomes the means of cure when it exists in the circulating blood." So of the twofold bactericidal and cytolytic agents we may say that the living body possesses substances which may protect it by destruction of invaders or may injure it by destruction of its own cells according to the mates with which these substances are joined.

An inquiry which naturally arises in this connexion is: What is the physiological mechanism called into action in the processes resulting in the production of antitoxins, cytolysins, and similar bodies? We have no reason to suppose that the animal body is endowed with properties specially designed to meet pathological emergencies. Its sole weapons of defence, often lamentably imperfect for morbid states, are adapted primarily to physiological uses.[4] To the foregoing inquiry Ehrlich answers that the mechanism concerned is one physiologically employed for the assimilation by the cells of food. The receptors are in the cells, not for the purpose of linking poisons to the cells, but to seize certain food stuffs, particularly the proteids, and the toxins and bacterial and other foreign cellular substances, if capable of inducing the immunizing reaction, chance to have the requisite combining affinities for the food receptors. It is interesting that Metchnikoff also, though from a different point of view, refers the mechanism of immunity to the physiological function of assimilation of food by the cells.

Inasmuch as, according to Ehrlich's hypothesis, the specific antagonistic substances resulting from the injection of toxins and of foreign cells or derivatives of cells exist preformed in cells of the normal body, there would appear to be no reason why any one of them might not occasionally be present normally free in the blood or other fluids. In fact, many of them —such as diphtheria and tetanus antitoxins, various antienzymes, bactericidal, haemolytic and other cellular toxins, agglutinins and a number of other bodies of this class, as well as their antibodies—have been found repeatedly, though of course in the case of many inconstantly and with marked differences between individuals and species, in the blood of healthy human beings or animals when their presence could not reasonably be attributed to a previous specific immunization. Of these normal antibodies the only one which is increased in amount by the process of immunization is that specifically related to the material used to bring about the reaction. As already stated, it is the intermediary body,[5] not the complement, which is generated in immunization against bacteria and other cells.

The foregoing statements, though of necessity condensed and incomplete, about the general characters of the specific antibodies will, I trust, help to a better understanding of what is to follow concerning the bearing of some of these discoveries on medical science and practice. I realize the difficulties which you must already have experienced, if unfamiliar with these new lines of research, in following a brief presentation of a subject in which not only are the facts so complex, and the ideas so novel, but the terminology so strange and burdened with such a multitude of confusing synonyms. While deploring the multiplication of unnecessary new terms, I should like to quote in this connexion a wise remark of Huxley:[6]

> If we find that the ascertainment of the order of Nature is facilitated by using one terminology, or one set of symbols, rather than another, it is our clear duty to use the former; and no harm can accrue so long as we bear in mind that we are dealing merely with terms and symbols.

The most remarkable and characteristic attribute of these antibodies is the specificity of their relation to the substances which have led to their formation. Of some of them, such as diphtheria or tetanus antitoxin, this specificity is nearly absolute; of others, such as the precipitins, it is only relative. This property is the basis of new and most valuable methods for the identification of species and the determination of genetic relations—species not only of living things, but also of chemical substances and of disease.

[4] W. H. Welch, Adaptation in Pathological Processes, *Trans. Congress American Physicians and Surgeons*, 1897, vol. iv.

[5] I use in this lecture the name "intermediary body" in preference to the more technical term "amboceptor," although Ehrlich applies the German equivalent—*Zwischenkörper*—only to normal as distinguished from immune amboceptors.

[6] Huxley, On the Physical Basis of Life. *Collected Essays*, Vol. i, p. 164. New York. 1893.

The resemblances and the differences thus revealed are doubtless fundamentally of a physico-chemical nature, but in many instances they transcend the powers of the microscope or of ordinary chemical tests to detect.

The results already attained by the method of serum diagnosis[7]—using this expression in its widest sense—are not only of interest and importance to the biologist, physiologist, and chemist, but of great practical value to the bacteriologist and the physician. As this is not an aspect of my subject, broad and important as it is, upon the details of which I propose to dwell, it must suffice to present by way of illustration examples of the diagnostic application of different kinds of specific serums.

The only certain means of detecting toxins of the class of diphtheria or tetanus toxin, snake venom, and certain vegetable poisons of the same category is their neutralization by the corresponding antitoxic serums. Occasion may arise where such detection is of practical and even medico-legal importance, as has been exemplified in India, where the criminal use of cobra venom is not unknown.

The application of serum diagnosis which is most familiar to physicians is the agglutinative test for typhoid fever. The principles of the agglutinative reaction were worked out in the laboratory of Professor Gruber in Vienna by himself and Durham, and were there first applied to the diagnosis of typhoid fever by Grünbaum, who was anticipated in his publication by Widal, who has made a thorough clinical study of the subject. The method is of great value, not only in the diagnosis of disease but also in the identification of bacterial species and the recognition of relationships between species. Durham, to whom we owe important contributions to this subject, has given an ingenious hypothetical explanation of mutual agglutinative reactions, the main features of which are paralleled in Ehrlich and Morgenroth's doctrine, based upon experiments, relating to the multiplicity of cell receptors and of amboceptors concerned in haemolysis.[8]

We have found the agglutinative reaction an indispensable aid in the study of the series of cases of paratyphoid fever which have come under observation in Dr. Osler's wards at the Johns Hopkins Hospital, and which otherwise it would have been scarcely possible to have separated from typhoid fever.[9] The occurrence of paratyphoid fever as a distinct disease affords an explanation of a certain proportion of the failures of the serum from supposed typhoid fever patients to clump typhoid bacilli. Not less valuable is the serum test in the diagnosis of bacillus dysenteriae (Shiga) and of the diseases caused by it. This micro-organism has been shown by Flexner and his pupils, Vedder and Duval, to be the cause of our acute dysenteries, and recently in Baltimore Duval and Bassett, working with the aid of the Rockefeller Institute for Medical Research at the Thomas Wilson Sanatorium for Children, have discovered that this same bacillus is in all probability the specific agent of infection in the summer diarrhoeas of infants.

Bacteriolytic serums have been used by Pfeiffer in the differentiation of cholera and allied spirilla, but few other bacteria present equally well the Pfeiffer reaction, which is not nearly so useful or handy a means of identification as the Gruber-Durham reaction.

Of other cytolytic serums the haemolysins have been by far the most carefully studied. One of the most interesting results of this study has been the determination by precise quantitative methods of resemblances and of differences between red blood corpuscles which in no other way could be dis-

[7] The general procedure followed in the production of specific serums is the injection into a suitable animal at intervals of time repeated doses of toxins, bacteria, foreign cells, or other material against which the antibody is desired. For example, if a specific precipitating or a haemolytic serum for human blood is wanted, an animal, say a rabbit, is injected subcutaneously or intraperitoneally at intervals of three or more days with five or six doses of human serum or human red blood corpuscles. At the end of this time the rabbit's serum in strong dilutions has acquired the property of precipitating human serum, or of dissolving human red blood corpuscles, if these were used for the injection. Within limits the less closely related the two species of animals the more powerful is the antagonistic effect of the specific serum. This is true especially in the case of cytotoxic serums.

[8] Durham, *Journal of Experimental Medicine*, January 15th, 1901, vol. v, p. 353. Ehrlich and Morgenroth, *Berl. klin. Woch.*, May 27th, 1901, p. 570.

[9] See papers on Paratyphoid Fever, by Johnston, Hewlett, and Longcope in *American Journal of Medical Sciences*, August, 1902.

tinguished. These resemblances and differences relate to the red corpuscles not only of different species of animals, but also to those of individuals of the same species. Although we constantly assume the existence of cellular differences between individuals and between species, these are for the most part of so subtile a nature as to elude our methods of observation. The exact demonstration of such differences by the use of cytolytic serums is therefore of especial interest. My assistant, Dr. H. T. Marshall, in an unpublished research, conducted under the direction of Professor Ehrlich and Dr. Morgenroth, upon the receptors of the red blood corpuscles of man and of two species of monkey, found that while man and the monkeys each have receptors not shared by the other, they also have a large number of receptors in common.

This result is in harmony with Nuttall's interesting observations on a much more extended scale regarding phylogenetic relationships between animal species, as shown by the reaction of their blood with the specific precipitins discovered by Tchistowitch and Bordet, and introduced into practical medicine by Wassermann. This biological test to determine the source of blood, when used with proper precautions, far surpasses in accuracy all other methods for this end. While it would lead too far from my purpose to follow this subject farther, I cannot in this connexion forbear at least mentioning one of the earliest and most suggestive papers on this class of antibodies—that On Immunity against Proteids, by Walter Myers, who gave up his life in the cause of science and of humanity, and whose early death is so great a loss to English medical science.

I shall ask your attention now to some considerations concerning the bearing of recent studies of immunity on the nature and action of toxins. This subject is, of course, of the greatest pathological as well as bacteriological importance, and I believe a closer co-operation than now exists between bacteriologists and pathologists in its study would further the surer and more rapid advancement of our knowledge about it. One misses only too often in purely bacteriological papers on this subject exact knowledge and descriptions of pathological conditions, and, on the other hand, pathologists often fail to utilize pertinent facts and ideas which are familiar to bacteriologists.

The discovery by Roux and Yersin of the diphtheria toxin, the studies by Behring and Kitasato of tetanus toxin leading up to Behring's epochal discovery of antitoxin, and the later investigations of Ehrlich on the constitution of diphtheria toxin and the origin and mode of action of antitoxin are the great events in the most brilliant and securely founded chapter of modern studies of immunity. Through these researches we became acquainted with a class of poisons secreted by certain bacteria, and present in solution in culture fluids. The evidence is conclusive that these soluble toxins enter, as assimilable substances, into direct combination with constituents of the body cells for which they have an affinity, and only thereby are enabled to bring about immunity or to exert toxic effects. As shown by the modifications of toxins called toxoids, the toxic property may be destroyed without loss of the combining power, and without removal of the immunizing power. According to Ehrlich's helpful conception, based on a large amount of experimental evidence, and now very generally accepted, the combining power of the toxin molecule resides in a group of atoms, designated as the haptophore group, with affinity for the corresponding haptophore groups of the side chains or receptors of cellular constituents, and the toxic power pertains to another and less stable atom complex in the molecule.

By means of these facts, and legitimate deductions from them, we are enabled to explain in a satisfactory way susceptibility to poisoning by these soluble toxins, their selective action upon the cells of the body, and their quick disappearance after injection into the circulating blood. In one infectious disease and in one only, to wit tetanus, are we able to explain the clinical and pathological phenomena in minute detail on the basis of our knowledge of the causative microorganism and its poisonous products. The nearest approach to this instance is diphtheria, but here we have not yet been able to follow the trail of the toxins within the body so perfectly, and, as Flexner and I have shown, in addition to the soluble toxins there is an intracellular poison concerned in the production of the false membrane. Interesting investigations, which have greatly helped to elucidate the nature of these toxins, have been made on various similar vegetable and animal poisons, such as ricin and abrin from the former source and the venom of snakes, spiders, and other poisonous animals.

The high hopes which were raised by the discovery of the soluble bacterial toxins that at last the way was opened for us to penetrate into the mysteries of the mode of action of pathogenic bacteria were soon doomed to disappointment, for similar powerful toxins, though diligently sought, could not be detected in the cultures of most other bacteria, and these among the most important ones, such as the tubercle bacillus, the typhoid bacillus, the cholera spirillum, the pneumococcus, the pyogenic micrococci. This disappointment was all the more acute because there was and is every ground for confidence that whenever we have in our possession a powerful toxin of this class, a strong protective antitoxic serum can readily be obtained.

Notwithstanding these negative results, the belief was not abandoned that bacteria harm the body mainly by poisoning, for it rests upon strong clinical and pathological evidence, as well as upon the study of the distribution of bacteria in the infected body. The search for poisons was turned from the fluid part of cultures to the bacteria themselves, and thus Pfeiffer succeeded in demonstrating as an integral constituent of the bodies of cholera spirilla toxic substances, which are liberated only when the bacteria degenerate or die. Intracellular poisons, which indeed previously, though of a different nature, had been extracted from bacteria by Buchner and by Koch, were subsequently found within typhoid bacilli and a number of other pathogenic bacteria.

It is of more than purely bacteriological interest to recognize the distinction between the small group of pathogenic bacteria, represented by the bacilli of tetanus, of diphtheria, and of botulism, characterized by the secretion of powerful soluble toxins, and the much larger group, containing most of the other pathogenic bacteria, which do not secrete similar strong toxins, for it is only the former which give rise to the production of antitoxic serum of marked protective and curative power.

The form of immunity resulting from injections or natural infections with the second class of bacteria belongs mainly to the bacteriolytic type, in which the complete antibody is not a single substance like antitoxin, but is composed of two distinct elements, intermediary body and complement, of which only the former is produced or increased in the process of immunization. The bacteriolytic serums are also under suitable, but not readily controlled conditions, protective and curative, but owing, it would seem, mainly to the duplex nature of the antibody their successful therapeutic application meets difficulties which have not yet been overcome. The great practical problem of bacteriology to-day is to make available to medical practice the bacteriolytic serums, such as antityphoid, antipneumococcus, antistreptococcus, antiplague, antidysentery serums. Such work as that of Marmorek, of Wassermann, of Neisser and Wechsberg, of Ainley Walker, upon the production, the properties, the conditions underlying the action of these serums is, therefore, highly important.

Our knowledge of the constitution and action of the intracellular bacterial poisons is most incomplete and at present cannot be applied in any very definite and satisfactory way to the explanation of the morbid phenomena of infectious diseases. Such investigations as those undertaken by Macfadyen and Rowland at the Jenner Institute of Preventive Medicine upon the expressed juices of bacterial cells promise to shed light upon this subject and in general upon the vital processes of bacteria.

I find it difficult to reconcile myself to the doctrine that bacteria, such as the typhoid bacillus, the pneumococcus, and others of the class now under consideration, do their chief injury to the body, not while they are lively and vigorous, but after they become corpses and in consequence set free their protoplasmic poisons. Still this latter conception is the basis of a coherent hypothesis of infection, elaborated most fully recently by Radziewsky,[10] which rests upon a considerable amount of accurate observation and interesting experi-

[10] Radziewsky, *Zeitschrift für Hygiene*, 1900, xxxiv, p. 185, and 1901, xxxvii, p. 1.

mental work. There can be no doubt that in the course of many infections there goes on an enormous destruction of the bacteria concerned, so that the numbers of those indicated at any given time by microscopical examination and by cultures may represent only an insignificant fraction of the total progeny of the first invaders. I have been much interested in this phenomenon, since I became familiar with it over twelve years ago[11] in pneumococcus infections through the employment of a method which revealed in the exudates degenerating and dead pneumococci and their empty capsules in numbers often far exceeding the intact organisms; indeed, in some cases so many that they formed a large part of the exudate.

While all due weight should be given to such facts as these, the objections to the acceptance of the hypothesis just mentioned as affording a complete explanation of the toxic phenomena of this class of infections are so obvious that naturally efforts have been made to learn whether bacteria which produce no strong soluble toxins in our ordinary culture media may not do so on other media of special composition or in a demonstrable way within the living body. Work in these two directions has not been altogether barren, as shown by results of experiments made by Hueppe, Cartwright Wood, Marmorek, and others along the former lines, and by Metchnikoff, Roux and Salimbeni along the latter, but it cannot be said that these experiments have led to any generally accepted solution of the main difficulties. Some are therefore inclined to lay the chief emphasis upon disordered cellular metabolism, but this is only a restatement of the question. Everybody recognizes abnormal metabolism as an essential condition in infections. The very point needing explanation is how the bacteria derange metabolism.

I wish here to advance a hypothesis which seems competent to explain the source, the mode of production, and the nature of certain bacterial toxins. It would appear to be a natural inference from the receptor theory of Ehrlich and the recent work on cytotoxins. The following considerations will, I hope, make clear the essential points.

As I have already stated, we know that the injection of foreign cells, such as pathogenic bacteria, red blood corpuscles, spermatozoa, epithelium, into the tissues of an animal leads to the formation of poisons, called cytotoxins, acting specifically upon these cells; that the substances which stimulate the cells of the host to produce one constituent of this class of toxins consist of certain atom complexes derived from the injected cells; that certain cells of the host thus stimulated generate and discharge one component of the toxin, called the intermediary body, which, although by itself not poisonous, becomes the medium of intoxication through union, on the one hand, with a pre-existent toxophore substance, called the complement, and, on the other hand, with the foreign cell which started the reaction.

Such is the response on the part of the host to the entrance of the foreign cells; but how about a possible response of a like nature on the part of the invading cells toward the host, resulting in the production of special cytotoxins, of analogous constitution, injurious to the host? This latter response, being of a vital nature, can take place only when the invading cells are living, as in the case of bacteria and other parasites.

I see no reason why suitable substances derived from the host may not stimulate parasitic organisms, through a physiological mechanism similar to that operative in the development of cytolytic immunity, to the production of intermediary bodies which, if provided with the requisite affinities, have the power to link complements to cellular constituents of the host, and thereby to poison the latter. Expressed in terms of Ehrlich's side-chain theory, certain substances of the host of cellular origin, assimilable by the parasites through the possession of haptophore groups with the proper affinities, become anchored to receptors of the parasitic cell, which, if not too much damaged, is thereby stimulated to the overproduction of like receptors; these excessive receptors of the parasite, if cast off into the fluids or the cells of the host, there constitute intermediary bodies or amboceptors with special affinities for those cellular constituents or derivatives of the host which led to their production, and for others which possess in whole or in part identical receptors. Provided the host is supplied also with the appropriate complements, there result cytotoxins with special affinities for certain definite cells or substances of cellular origin in the host. The contribution of the parasitic cells to these cytotoxins is the amboceptors. Either the parasite or the host may provide the complements.[12]

It may perhaps aid in grasping the ideas here presented to imagine the bacterium, in the capacity of the host, as a structure so large that one could inject into it animal cells. Provided the proper receptor apparatus is present, the resulting reaction on the part of the bacterium, as described, would be a process of immunization against the animal cells through the formation of specific cellulicidal substances. In reality it is only certain atomic complexes of cells which are concerned in this immunizing reaction, and in comparison with these even the smallest bacterium is a gigantic object.

Looked at from the point of view of the bacterium as well as from that of the animal host, according to the hypothesis advanced the struggle between the bacteria and the body cells in infections may be conceived as an immunizing contest in which each participant is stimulated by its opponent to the production of cytotoxins hostile to the other, and thereby endeavours to make itself immune against its antagonists. These mutually antagonistic cytotoxins are capable of injuring the parasitic cells on the one hand or the body cells on the other, only when escaping combination outside of them they are anchored to the receptors of the cells to which their respective affinities are adjusted. This combination with the cells, if it does not result in too great injury to them, is the condition for further production of the cytotoxic intermediary bodies through over production and discharge of receptors.[13] The important factors determining the issue of the contest are the relative proportions and the distribution of the bacterial and the host's cytotoxins.

The hypothesis thus outlined can be tested experimentally, but I regret that it has shaped itself in my mind so recently that I have not yet been able to make the desired experiments, which are, however, now started in my laboratory. Since my arrival here I am informed that these experiments have already furnished facts in its support, which will be published later.

Inasmuch as at least one component, and it may be both components, of the assumed bacterial cytotoxins pre-exist in the bacterial cells, it should be possible to demonstrate some of them in artificial cultures of bacteria, where they would be found especially as integral parts of the cells,

[11] Welch, *Bulletin of the Johns Hopkins Hospital*, July, 1890, and December, 1892. Michaelis, *Berl. klin. Woch.*, 1902, No. 20, has recently reported the same findings.

[12] We may thus speak of somatogenic cytotoxins resulting from the action of bacterial stimuli on cells of the host, and of bacteriogenic cytotoxins from somatogenic stimuli, also of somatogenic and bacteriogenic complements.

[13] It will be observed that these discharged receptors may be regarded as the equivalents of anti-immune bodies. E. W. Ainley Walker, in an interesting and suggestive paper on Immunization against Immune Serum (*Journal of Pathology and Bacteriology*, March, 1902), shows experimentally that bacteria growing in immune serums produce anti-immune bodies, and thereby become more virulent. He concludes that "the basis of bacterial virulence and of chemiotactic influence is identical, and constitutes that atom group which causes the production of the immune body." My hypothesis includes the conceptions supported by Walker, and also much more. According to the hypothesis, certain bacterial antibodies are capable not only of neutralizing immune bodies of the host, but with the aid of complements also of poisoning the cells of the host. It is not difficult to imagine various conditions in which the antibodies of bacterial origin may escape neutralization before entering into union with the host's cells. The substances which stimulate bacteria to produce these antibodies need not necessarily be toxic to them; in fact, toxicity, such as that of strong bactericides of cellular origin, would hinder their production. The essential things are that the stimulating substances have the requisite combining groups for bacterial receptors, and that the cast-off receptors be complemented within the body of the host. Each of the various bacteriogenic cytotoxins probably contains a multitude of partial amboceptors, with varying cytophilic and complementophilic affinities, in accordance with the views of Ehrlich and Morgenroth. It is self-evident that through a mechanism similar to that described parasites within the infected body may be stimulated by atom groups derived from the host to the production also of antibodies other than cytotoxins, such as various agglutinins, precipitins, antienzymes, and perhaps of uniceptors of the nature of secreted soluble toxins, or of enzymes, all adjusted against the host. Questions relating to the source and nature of the complements, particularly of intracellular complements, and also to anticomplements, are manifestly of importance in relation to the hypothesis, but it would complicate the subject too much to discuss these and other matters here, where my purpose is merely to outline the essential features of this new theory of infection with reference to the particular points under consideration.

unless extracted from the bodies of degenerating or dead bacteria. This corresponds with what is known concerning the situation of the poisons of the cholera spirillum, the typhoid bacillus, and other bacteria characterized by the lack of strong soluble toxins. But the quantitative and other relations between these cultural cytotoxins and those produced in the manner described by the same bacteria during processes of infection are comparable to those between the normal antibodies and the immune antibodies. These relations would explain the familiar fact that cultures of bacteria of the class under consideration constitute in general only a partial and meagre index of the toxic capacities of the same bacteria in the infected body. That cytolysins may, however, be present normally in large amount is illustrated by the haemolysins of eel's serum and of snake venom.

In this theory, degenerated and dead bacteria, while recognized as a source of poisoning in infections, are not assigned an exclusive rôle in this regard. Living bacteria in the infected body, where they are under nutritive conditions not paralleled in artificial cultures, actively produce and secrete receptors which may become the means of intoxication of the body cells. From what has been said, we can comprehend how these diverse free receptors may enter into the formation of cytotoxins of the most varied and specific characters, such as erythotoxins, leukotoxins, neurotoxins, nephrotoxins, spermotoxins, hepatotoxins, etc. Very probably in many instances these toxins are represented by so few receptors in bacterial cells in ordinary cultures that it would be hopeless to search for them there, although we may have convincing experimental and pathological evidence that within the animal body the same bacteria produce them abundantly under the stimulus of appropriate substances derived from cells of the host.

The methods hitherto employed for the study of bacterial poisons have not generally been calculated to reveal the presence of toxins with the characters indicated, even if such existed in the cultures. Recently, however, a beginning has been made in this direction, and we have already become acquainted with certain toxins of an interesting nature, to which I desire to direct your attention.

Intrinsically and in their general bearing upon the subject before us, the recent investigations of Flexner and Noguchi upon the constitution of the toxins in snake venom are of especial importance. It was in snake venom that Weir Mitchell and Reichert first demonstrated the existence of that class of poisons often called, although with doubtful propriety, toxic albumins. Investigations of snake toxins are of peculiar interest for many reasons, not the least of which is their resemblance to bacterial toxins. The demonstration by Sewall of the possibility of active immunization from venom, and the further studies by Calmette and by Fraser of this phenomenon, and especially of the protective and curative properties of antivenene are well known.

Until recently it has been generally held that the venom toxins resemble in molecular structure the diphtheria and the tetanus toxins in being single bodies with a combining or haptophore group and a toxophore group of atoms. The researches of Flexner and Noguchi, now in progress, of which only the first part has been published,[14] necessitate a quite different conception of the nature and manner of action of venom toxins from that previously entertained. I have followed with great interest the work of Professor Flexner on toxins, begun several years ago in my laboratory when he was my assistant and associate, and since continued along new lines in his laboratory at the University of Pennsylvania, and I wish to acknowledge his generosity in permitting me to use in this lecture certain unpublished results of his and Noguchi's investigations.

These investigations have shown that the toxic action of venom upon red blood corpuscles, leucocytes, nerve cells, and other cells is like that of the duplex cytotoxins already described—that is, it depends upon the combination of intermediary bodies contained in the venom, on the one hand with the animal cells for which these bodies respectively have affinities, and on the other hand with corresponding complements contained, not in the venom, but in the cells or fluids of the animal acted on. For example, it is well known that the addition of venoms to fresh blood brings about the quick destruction and solution of the red corpuscles. If, however, certain venoms be added to red corpuscles which have been thoroughly washed with isotonic salt solution so as to remove all the complement, the corpuscles are agglutinated but not dissolved, although it can be shown that substances from the venom (intermediary bodies) have entered into combination with the corpuscles. If now a little fresh serum which contains the complement, and by itself may be an excellent preservative of normal corpuscles, be added to these venomized corpuscles, they are promptly dissolved.

Preston Kyes, working in Professor Ehrlich's laboratory, in an investigation just published[15] on the mode of action of cobra venom, confirms the conclusion of Flexner and Noguchi concerning the amboceptor nature of cobra venom, and adds much that is new and important to our knowledge of this subject. He finds that the washed blood corpuscles of certain animals are directly dissolved by cobra venom, while those of other animal species require the subsequent addition of complements or adjuvants to bring them under the influence of the venom. But even in the former case a complementary body is essential to the reaction, this, however, being not a serum complement, but an endocomplement contained within the red corpuscles. Of great significance is the demonstration by Kyes of still a third substance, namely lecithin, which is capable through combination with the venom intermediary body of completing the haemolytic potency of venom.[16] The discovery for the first time of a definite, crystallizable substance with the power of uniting, like a complement, with an intermediary body, and thus completing the formation of a cytotoxin, is evidently of fundamental importance. The suggestion by Ehrlich and Kyes that possibly the cholin group is the toxophore group of lecithin is particularly interesting in the light of F. W. Mott's valuable studies of chemical processes concerned in degenerations of the nervous system.

The researches of Flexner and Noguchi and of Kyes, therefore, have taught us that in poisoning by venom the bodies of human beings and of animals contain in the form of complements, or alexins[17] as they are also called, the substances which are most directly concerned in the act of poisoning. The venom serves merely to bring into the necessary relations with constituents of the body cells poisons we already harbour or may generate, but which are harmless without the intervention of intermediary bodies. These poisons within us are powerful weapons, which when seized by hostile hands may be turned with deadly effect against our own cells, but which are also our main defence against parasitic invaders. We see here as everywhere that Nature is neither kind nor cruel, but simply obedient to law.

Flexner and Noguchi have demonstrated experimentally that, like the haemolytic, so also the leukotoxic, the neurotoxic, and other cytotoxic properties of venom depend upon combinations of venom intermediary bodies with complements contained in the cells poisoned by venom or in the fluids bathing these cells. Particularly striking are their experiments showing *in vitro* and under the microscope the cytolytic action of cobra venom upon certain large molluscan nerve cells in the fresh state. The complement essential to this reaction is contained within the nerve cells. In previous experiments of Flexner and Noguchi there had been indications that a special class of intracellular complements are concerned in some of the toxic effects of venom upon cells. The positive demonstration by Preston Kyes of a special class of intracellular complements or endocomplements is unquestionably of great pathological interest, and seems destined to play an important part in the explanation of many morbid

[14] Flexner and Noguchi, Snake Venom in Relation to Haemolysis, Bacteriolysis, and Toxicity, *Journal of Experimental Medicine*, March 17th, 1902, vol. vi, p. 277.

[15] Preston Kyes, Ueber die Wirkungsweise des Cobragiftes, *Berl. klin. Woch.*, 1902, Nos. 38 and 39. I am greatly indebted to my friend Professor Ehrlich and to my former pupil Dr. Kyes for putting me in possession of the main results of these interesting experiments before the date of their publication.

[16] The objections made by Calmette (*Compt. Rend. Acad. de Sc.*, 1902, T. cxxxiv, No. 24) to Flexner and Noguchi's interpretation of their experiments as to the amboceptor nature of venom have been completely overthrown by the experiments of Preston Kyes.

[17] There is some objection to the use of the term "alexin" as a synonym for "complement," as the former was applied originally by Buchner to substances which we now know to be combinations of complements with intermediary bodies.

conditions in connexion both with endogenic and with exogenic intoxications, probably also in such phenomena as self-digestion or autolysis.

Snake venom is a rich mine of diverse toxins, and, on account of its pathological importance, I must mention one of the cytotoxins discovered there by Flexner and Noguchi, as it may be that a similar toxin is produced by certain bacteria, and under still other conditions. As is well known, one of the most striking lesions resulting from poisoning by certain venoms is the occurrence of abundant haemorrhages in various tissues of the body. This effect has been generally attributed to the direct action of venom on the red corpuscles and on the coagulability of the blood, but the experiments of Flexner and Noguchi indicate that these haemorrhages are due to the presence in venom of a cytotoxin which has the power of dissolving endothelial cells—in other words, an endotheliolysin. Dr. Flexner suggests the name "haemorrhagin" for this special toxin which causes extravasations of blood through its direct solvent action upon capillary endothelium, an effect which is readily demonstrable under the microscope. It is hardly necessary for me to stop to emphasize the clinical and pathological importance of the discovery of an endotheliotoxin, a kind of poison which may prove to be of special significance in the interesting group of haemorrhagic infections, and perhaps also in purpura and kindred affections.

The foregoing newly-discovered facts, which I have sketched only in bare outline, illustrate in a striking way the fruitfulness of methods and conceptions which we owe to recent studies of immunity. The results of these investigations, however, are significant beyond the mere facts disclosed, important as these are. They have for the first time revealed in normal toxic secretions, readily introduced under conditions of nature into the tissues of man and animals, cellular poisons akin to the complex haemolysins, neurotoxins, and other cytotoxins of immune and some normal serums, which have aroused so much interest and experimental study during the past four years. The most noticeable difference between the venom cytotoxins and those hitherto observed in immune serum is the far greater resistance to heat of the intermediary bodies of the former; but we are already acquainted with considerable variations in the sensitiveness to heat both of different intermediary bodies and of complements. That snake venom should contain only one half of the complete poison, the other and the really destructive half being widely distributed in the blood and cells of man and of animals, is an instance of a curious kind of adaptation, of interest from evolutionary, as well as from other points of view.

In consideration of the often emphasized analogies between venom toxins and bacterial toxins, these facts render it highly desirable to make a systematic search of bacterial cultures by proper methods and under suitable conditions for complex cytotoxins. At present substances of this nature are not known to exist in our cultures. There have been discovered, however, within the past three or four years certain bacterial toxins which have a curious resemblance in some of their properties to the complex antibodies of blood, although, so far as they have been carefully studied, they appear to have the simpler constitution of the soluble toxins, like those of diphtheria and of tetanus. I refer to the bacterial haemolysins, leukolysins, haemagglutinins, precipitins, and coagulins. There is no reason to suppose that this list exhausts the number of those actually present, for it is evident that it includes chiefly bodies readily demonstrable in test-tube experiments. It would be surprising if cytotoxins which act specifically upon red and white corpuscles were the only ones of this class produced by bacteria; in fact, we have every reason from pathological observations to believe the contrary.

It has become evident that more refined methods than mere observation of the coarse effects of injecting into animals the filtrates or the killed bacteria of our cultures are required for the detection of the subtler and more specific cellular poisons. Instances are rapidly increasing in which by improved methods cultures of bacterial species once believed to be practically devoid of toxicity are found after all not to be so poor in toxins, even of the soluble variety. One of the earliest and most instructive illustrations of this is the discovery by Van der Velde of a leucocyte-destroying poison, named leucocidin, in exudates caused by infection with staphylococcus aureus, and also in filtrates of staphylococcus cultures, which had been previously regarded as almost entirely free of toxic power.

More widely distributed in cultures of different species of bacteria are the haemolysins, of which the first example, discovered in 1898 by Ehrlich in cultures of the tetanus bacillus, was carefully studied by Madsen the following year, and which have since been investigated by Kraus with Clairmont and with Ludwig, Bulloch and Hunter, Neisser and Wechsberg, Todd, Besredka, and others. The list of bacterial species known to produce in cultures substances of this nature capable of dissolving red blood corpuscles is already a long one, and includes the bacilli of tetanus, of green pus, of typhoid fever, of acute dysentery, of diphtheria, of plague, the pyogenic staphylococci and streptococci, the pneumococcus, and many other bacteria. Nuttall and I noted in our first description of bacillus aërogenes capsulatus over ten years ago its capacity of laking blood, so that I was not surprised to find recently that a haemolysin can be demonstrated in cultures of this organism. The blood-destroying property appears to stand in no definite relation to virulence, nor is it limited to pathogenic bacteria. It pertains also to many putrefactive bacteria. The strongest bacterial haemolysin hitherto observed was found by Todd in cultures of bacillus megatherium, which is a widely distributed saprophyte.

As already stated, none of these bodies has been shown to belong to the class of complex haemolysins in blood, which have been far more exhaustively investigated than any other of the specific antibodies. Doubtless there is at present among bacteriologists too great a tendency to attribute to the less carefully studied antibodies characters which have been worked out in detail only for the haemolysins of immune serum. It would lead too far to attempt here a discussion of the special characters of the various bacterial haemolysins, which present in different specimens curious and at present unexplained divergences as regards resistance to heat and several other properties. It must suffice to indicate briefly what is known of the pathological importance of this interesting group of bacterial toxins.

In view of the abundant clinical and pathological evidence of extensive destruction of red corpuscles in the course of many infectious diseases, it is certainly significant to find that many bacteria are endowed with a specific haemolytic power. The question is how far we are justified in applying to the actual conditions of infection the existing experimental data upon this subject. Assuredly here, as everywhere, results of test-tube experiments, helpful in suggestion as they may be, should not be utilized without further evidence to explain morbid phenomena within the infected human or animal body. While much more work upon this subject is needed before our information will be exact or complete, the observations and experiments of Besredka,[18] Kraus and Ludwig,[19] and others have already demonstrated that bacteria may exert their blood-destroying power within the living body. This haemolytic capacity of micro-organisms affords an explanation, although certainly not the only one, of the secondary anaemias which are such a marked feature of many infectious diseases, as streptococcus and other septicaemias, pneumonia, typhoid fever, scarlatina, and others. The haemoglobinuria which is a recognized although rare complication of various infectious diseases may be referable to intoxication with unusually powerful bacterial haemolysins, or to an exceptional lack of resistance of red corpuscles.

Haemoglobin, however, is not necessarily present in solution in the blood plasma, for the destruction of the damaged red corpuscles may take place within the large phagocytes of the spleen and the haemolymph glands, as is well known to occur on an extensive scale in typhoid fever and some other infections. A familiar example of the action of bacterial haemolysins is the *post-mortem* reddening of the inner lining of the heart and blood vessels, an effect which may be due to putrefactive bacteria or may appear very soon after death, especially from septicaemia caused by streptococcus

[18] Besredka, *Annales de l'Institut Pasteur*, 1901, xv, p. 880.
[19] Kraus and Ludwig, *Wien. klin. Woch.*, 1902, p. 382.

pyogenes, which, as has been shown, may lake the blood during life.

The fact that certain common saprophytic bacteria may produce energetic haemolysins, as pointed out by Kraus and Clairmont and by Todd, has a possible bearing upon the etiology of certain obscure anaemias not of infectious origin, particularly upon the interesting observations and the theory of William Hunter concerning their causation by absorption of toxins from the alimentary tract. Todd found cultures of bacillus megatherium so strongly haemolytic that the intravenous injection of 1 c.cm. of the filtrate into guinea-pigs was followed by haemoglobinuria, 10 c.cm. being fatal. Human red corpuscles are sensitive to this haemolysin.

Normal human and other blood serums contain in varying amounts antihaemolysins, which protect the red corpuscles from the action of some of the bacterial haemolytic agents. Specific antihaemolysins are readily produced by immunizing injections of bacterial haemolysins, and are generated also in the course of infections. Lang suggests that the augmentation of the osmotic resistance of the erythrocytes which has been noted in some infectious diseases, as well as in icterus and some other morbid conditions, may be a reactive phenomenon caused by the presence of haemolytic toxins.

Intimately associated with the haemolysins in cultures are the bacterial haemagglutinins,[20] substances which have the power to clump red blood corpuscles. Among unicellular organisms both the capacity to produce agglutinins and the aptitude for agglutination seem to be very widely distributed. The bacterial haemagglutinins, in analogy with the bacterial haemolysins, are apparently of simpler constitution than the serum agglutinins, being destroyed at 58° C., whereas the latter are not injured by temperatures under 70° C. In order to demonstrate in cultures the haemagglutinins it is generally necessary to eliminate in some way the action of the associated haemolysins, which can be done by using small quantities of the culture fluid or by keeping the mixture of fluid and red corpuscles at zero temperature.

I know of no observations directly demonstrative of the action of bacterial haemagglutinins within the living body in infections, but this subject is of such recent knowledge that it has been as yet scarcely investigated. Certainly there are morbid conditions which seem highly indicative of the operation of substances agglutinative of red corpuscles. Probably every one with large experience in the examination of fresh blood in disease has noticed that sometimes red corpuscles, examined immediately after withdrawal of the blood, have a peculiar tendency to form clumps which cannot readily be broken up. This phenomenon, which is certainly suggestive of the action of an agglutinating agent, I have observed especially in some cases of septic infections and of cirrhosis of the liver.

Furthermore, I would emphasize the support given by the recognition of haemagglutinins to views advocated many years ago by Hueter and by Klebs concerning the occurrence of thrombi composed of coalesced red blood corpuscles. Such thrombi I believe to be not uncommon in typhoid fever and other infections, especially in small blood vessels. I have elsewhere called attention to the evidence in favour of the interpretation of many of the hyaline thrombi as derived from agglutinated red corpuscles.

It can scarcely be doubted that substances agglutinative of white blood corpuscles are also produced by certain bacteria, and that these are concerned in the clumping of pus cells and of leucocytes within the living body, but it would not be profitable to discuss this matter without more exact information than we now possess.

In this connexion I may say that not only the discovery of the bacterial haemagglutinins, but also that of the haemolysins and the leukolysins, is likely to shed new light upon certain aspects of the difficult subject of thrombosis. The red corpuscles undergo various morphological changes under the influence of different bacterial haemolysins acting with varying intensity. Distortions of shape, throwing out of projections, and detachment of colourless particles resembling platelets, can sometimes be seen. These observations are of especial interest with reference to the doctrine, already strongly supported, that platelet thrombi originate from disintegrated red corpuscles. Levaditi, and Neisser and Wechsberg, have described, as the result of intravenous injections of staphylococcus aureus, areas of coagulative necrosis in the rabbit's kidney, which they attribute to thrombi composed of disintegrated leucocytes caused by the staphylococcus leucocidin, to which I have already referred.

I have dwelt at some length, although of necessity incompletely, upon the bacterial haemolysins, leucocidins, and haemagglutinins, because we are better informed about these agents than concerning other members of this recently recognized class of bacterial toxins. I have already expressed the opinion that similar poisons acting specifically upon other cells of the body are produced by bacteria; indeed neurotoxins and nephrotoxins of this type have been reported. The difficulties in the way of direct proof of the existence of these other bacterial cytotoxins are greater than in the case of those acting upon the red and the white blood corpuscles, but doubtless they can be overcome. Of course we have evidence of the action of bacterial poisons upon various body cells, but this is not enough. At present we can apply only in a vague and unsatisfactory way to the explanation of pathological processes most of the knowledge of this kind which we possess. What is urgently needed is a separation of these poisons and a determination of their source, constitution, mode of action, and degree of specificity along such lines as have been followed so fruitfully in the investigation of the soluble diphtheria and tetanus toxins, those other toxins of bacteria and of venom already considered, and the cytotoxins of normal and of immune serum. The path leading apparently in the right direction has already been opened, and, if I mistake not, its further pursuit is most promising of valuable results in the near future.

Consider by way of illustration how helpless we now are in our efforts to explain the characteristic lesions of typhoid fever on the basis of our knowledge of the properties of the typhoid bacillus. That these lesions are referable to the action of toxins cannot, I think, be seriously questioned. Especially from the investigations of Mallory, we know that the most characteristic histological changes of this disease consist in the proliferation of the reticular or so-called endothelial cells of the lymphatic tissue of the intestine and the mesenteric glands and of similar cells in the splenic pulp, and in the assumption by these proliferated cells of remarkable phagocytic activities towards the lymphocytes in the former situations and towards the red corpuscles in the spleen. Mallory believes that these changes are best interpreted by supposing that the typhoid toxin directly stimulates to proliferation the endothelial cells, which then devour their offspring, the lymphocytes, and the red corpuscles.

I have suggested as another explanation that the typhoid bacillus produces a lymphocytotoxin and a haemolysin, and that the proliferation of the fixed cells is partly compensatory and partly for the increased production of macrophages. We already know that this bacillus generates a haemolytic agent, and we also know that one of the effects of injection of haemolysins is to increase greatly the number of macrophages containing red corpuscles in the spleen.

Through the kindness of Professor Flexner I have had the opportunity of studying the extraordinary changes produced in all the lymphatic glands and in the bone marrow of rabbits by injections of lymphotoxic or myelotoxic serum obtained by treating a goose with lymphatic or marrow tissue of the rabbit. One of the most striking effects of this poison for lymphocytes and other leucocytes is the very extensive proliferation of the reticulum cells in the lymphatic nodes and of the marrow cells. In the light of these observations it is clear that a positive demonstration of the production of a lymphotoxin by the typhoid bacillus would materially advance our understanding of the morbid anatomy of typhoid fever. Another lesion of this disease, only second in importance to those mentioned, is the occurrence of plugging of the small vessels. Dr. Fisher, in my laboratory, has recently shown that such thromboses are produced by the experimental inoculation of rabbits with the typhoid bacillus. I have already pointed out that many of these plugs are agglutinative thrombi.

Of course infectious diseases other than typhoid fever could also be cited, did time permit, as equally forcible illustrations of

[20] Kraus and Ludwig, *Wien. klin. Woch.*, 1902, p. 120.

the aid which pathology may reasonably expect from more precise knowledge of the bacterial cellular poisons. It is probable that such knowledge will lead to improvements in the quality for therapeutical purposes of the so-called bacteriolytic serums, some of which, as now prepared, are not so wholly devoid of antitoxic properties as is often represented. We may also anticipate from investigations of the character indicated much light upon one of the most puzzling of bacteriological problems—the localization of bacteria in disease. Toxic lesions and the plugging of small blood vessels are certainly often of decisive influence in determining this localization, as has been demonstrated especially for the staphylococcus pyaemias by Muscatello and Ottaviano.[21]

The toxins to which I have chiefly directed your attention in this lecture are those produced by bacteria. But, as already pointed out, we now know that the animal body has the power to produce specific poisons directed not only against invading bacterial cells, but also against all sorts of foreign cells. Following the discovery by Belfanti and Carbone in 1898 of this capacity in relation to injections of blood a wholly new domain of biology has been opened to experimental research. Attention has been withdrawn for the moment to a considerable extent from the bacterial toxins, and concentrated upon the animal cytotoxins. Here new facts and conceptions of absorbing interest have been disclosed in an abundance and with a rapidity which are simply bewildering.

It was my original design to include in this lecture a consideration in some detail of these animal cytotoxins, but so much time has been occupied with other aspects of the subject that I am compelled to abandon this intention. This is perhaps less to be regretted, inasmuch as I understand the main purpose of these lectures to be the presentation of applications to medicine and surgery of scientific discovery, and it is precisely this side of the recent work on animal cytotoxins which seems to me in the main not yet ripe for profitable discussion on this occasion. It is true that facts of much scientific and practical interest have been discovered by the investigations, initiated by Shattock and by Grünbaum, followed by Landsteiner, Ascoli, Eisenberg, Kraus and Ludwig, and others concerning the isoagglutinative and isolytic properties of human serums in health and in disease.

But the really great practical questions in this domain relate to the production of autocytotoxins in the human and the animal body. What is the nature of that very efficient regulatory mechanism underlying the horror autotoxicus (Ehrlich) which prevents either the action or the formation of autocytotoxins in consequence of absorption of our own degenerated and dead cells? Can this protective mechanism be overthrown by pathological states and self-generated cellular poisons become operative in the causation of anaemias, haemoglobinurias, chronic interstitial inflammations, uraemia, eclampsia, epilepsy, and other diseases? To these and similar important questions the existing experimental data seem to me too insufficient and inconclusive to furnish any decisive answer at present. I share, however, the hope and belief of many that here is a field for exploration which, although surrounded with many difficulties, gives promise of discoveries of a far-reaching and important nature. I anticipate that some future Huxley lecturer will find in this realm a fascinating theme.

In this connexion may be mentioned the great pathological interest pertaining to the recent investigations of Jacoby, Conradi and others on the phenomena of self-digestion or autolysis of inflammatory exudates and necrotic material within the living body. One can readily convince himself of the energetic action of autolytic ferments by the simple experiment of placing a piece of fresh pneumonic lung in the stage of grey hepatization under chloroform and noting the rapid solution of the exudate, in contrast with the absence of this process in earlier stages of the disease. Conradi finds that bactericidal substances, to which he attaches much importance, are produced in tissues and cellular exudates undergoing autolysis.

Although my theme relates especially to the bearing of studies of immunity on pathology, it is hardly necessary to say that these studies were primarily undertaken to elucidate the great problems of predisposition and resistance to disease, and that in this field they have borne their richest fruits. It is especially gratifying to note the close convergence of the two doctrines of immunity, the cellular and the humoral, brought about by these recent discoveries. On the one hand the phagocytic school, represented so brilliantly by Metchnikoff and his co-workers in the Pasteur Institute, recognize and apply to the fullest extent in the explanation of acquired immunity the cytolytic principles based upon the co-operative action of intermediary bodies and complements. On the other hand the humoral school, led by our German *confrères*, which has been so fruitful in results of the greatest scientific and practical value, recognize the cells, and especially the leucocytes and other cells of the blood-forming organs, as the immediate source of the protective substances. There are many differences in details, especially in terminology and in interpretation, which make the divergence seem greater than it really is. The essential difference between the two schools concerns the place where the two forces, intermediary body and complement, unite. All are agreed that the intermediary body exists free in the blood plasma, but Metchnikoff strenuously insists, especially on the basis of Gengou's experiments, that the complement or cytase is within the leucocytes, from which it is not secreted but can be liberated only through damage to these cells. This question certainly needs further investigation before it can be regarded as settled.

The deeper insight which we have recently gained into the nature of the forces concerned in immunity makes especially desirable the systematic study of the blood and other fluids of human beings in health and in disease with reference to their content of specific antibodies, particularly of the bactericidal substances. It can scarcely be doubted that knowledge of this kind will be in various ways of practical value to the physician and surgeon. The simplest procedure, and the one hitherto generally adopted, is the estimation of the bactericidal power of the blood serum *in toto*. For this purpose Professor Wright[22] has devised an ingenious method which in his hands has furnished extremely interesting information concerning variations in the bactericidal power of the blood as regards the typhoid bacillus in health, under the influence of fatigue, in the course of typhoid fever and after antityphoid inoculations. The older methods, however, while not without value, do not inform us of the total content of the blood in immunizing substances, and have led to very discordant results, particularly as to the influence of infection upon the bactericidal power. Thus Conradi[23] finds, in opposition to most previous experimenters as well as to the later results of Wilde, that infection with the anthrax bacillus does not at any stage influence materially the bactericidal properties of the blood.

A useful and readily applicable method for the determination separately of the intermediary bodies and of the complements of human serum is urgently needed. When one takes into consideration the plurality of complements and of intermediary bodies, the fallacies of interpretation which may arise from failure to take account of anticomplements, of antiimmune bodies, of complementoids, of amboceptoids, of deviation (*Ablenkung*) of complements, and other principles in this complicated subject, it is clear that the problem is not an easy one.

Notwithstanding these difficulties, work has already begun along these new lines, and has led to interesting results. We know that the content of the blood in specific antibodies, and especially in complements, varies in significant ways under diverse conditions, as in infancy and in adult life, in health, in different states of nutrition, under the influence of fatigue, of inanition, of pain, of interference with respiration, of alcohol, and in disease. The infant comes into the world with protective antibodies in the blood smaller in amount and less energetic than those possessed by the healthy adult. It is an important function of the mother to transfer to the suckling through her milk immunizing bodies, and the infant's stomach has the capacity, which is afterwards lost, of absorbing these substances in an active state. The relative richness of the suckling's blood in protective antibodies, as contrasted

[21] Muscatello and Ottaviano, *Virchow's Archiv*, 1901, clxvi, p. 212.

[22] A. E. Wright, *Lancet*, 1898, i, p. 95; 1900, ii, p. 1556; 1901, i, pp. 609 and 1532; and 1901, ii, p. 715.

[23] Conradi, *Zeitschrift für Hygiene*, 1900, xxxiv, p. 185; 1901, xxxviii, p. 411.

with the artificially-fed infant, explains the greater freedom of the former from infectious diseases.

The important question of the influence of pre-existent disease in predisposing to infection has been brought nearer to a solution by recent studies of immunity. Schütze and Scheller[24] have demonstrated that, while the normal rabbit promptly regenerates the complements used up in consequence of the injection of haemolytic serum, a rabbit infected with the hog cholera bacillus has lost this capacity. My former pupil, Dr. Longcope, has kindly placed at my disposal the unpublished results of an investigation which he is making under Professor Flexner's direction at the Pennsylvania Hospital of the intermediary bodies and the complements in human blood in different diseases. Colon and typhoid bacilli are used as the tests, as, unless one accepts Bordet's doctrine of the unity of complements, it is more important for the study of problems of infection to determine bacteriolytic rather than haemolytic antibodies. One of the earliest results of the systematic bacteriological examinations which we make at all necropsies at the Johns Hopkins Hospital was the recognition of the great frequency of terminal infections, formerly often undetected by the clinician, in chronic diseases, particularly of the heart, the blood vessels, and the kidneys. Dr. Longcope finds, although not regularly, still in many cases of these diseases a marked reduction in the quantity of complements, which may amount to a total loss of the colon complements. The analysis of the cases brings out unmistakably a definite relation between this loss of complement and the predisposition to infection.

The study of a series of acute infections, chiefly of a surgical nature, shows that in the course of the infection complements are being constantly used up and regenerated, and that at any given time there may be an excess or a reduction of the bacteriolytic power of the blood. Thus far it has been found impossible in these acute infections to attach any prognostic significance to the amount of complement or of bacteriolytic power, nor could any definite relation be determined between the leucocyte count and the content of complements.

Although we have traversed, gentlemen, in this lecture a path which I fear has seemed to you a long and winding one, I am conscious that I have been able to point out the features of the prospect only imperfectly and incompletely. The extent and the richness in details have been embarrassing. I trust, however, that I have been able to indicate in some measure the great interest and importance to biology, to physiology, to pathology, to every department of medical science and art of investigations which have led to a deeper insight into certain manifestations of cellular life. What has been conquered by these investigations is simply a bit of new territory pertaining to the intimate life of the cells, and we find here, as whenever we are able to penetrate deeper into this life, that there comes a flood of new light into every department of biology. The researches on immunity, which to some of short vision once seemed to threaten the foundations of cellular pathology, have served only to strengthen them. These researches, which have already led to the saving of thousands of human lives, and will lead to the saving of untold thousands more, have been carried on by the experimental method, and can be conducted in no other way. This method is as essential for the advancement of medical science as for that of any of the natural or physical sciences. To restrict unnecessarily and unjustifiably its use is nothing short of a crime against humanity. It is an evidence of the robust vitality of English physiology and medicine that in spite of unwarrantable obstacles thrown in their path their contributions to science in recent years have been so numerous and so important. The influence of English thought and action is great with us in America. See to it, my colleagues and men of science in the British Isles, that you retain for yourselves and hand down to your successors, at least without further impairment, the means of promoting medical knowledge and thus of benefiting mankind.

[24] Schütze and Scheller, *Zeitschrift für Hygiene*, 1901, xxxvi, pp. 270 and 459.

CONCERNING SPASTIC AND SYPHILITIC SPINAL PARALYSIS.*

By Professor Dr. WILH. ERB,
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Gentlemen,—I consider it a great honour to be allowed to deliver a lecture in this important institution, which is consecrated to medical learning and practice, and before an assembly of young colleagues all eager for knowledge. I respond with joy and thankfulness to the invitation, and hope that I shall be sufficiently understood, in spite of my imperfect English, and perhaps unaccustomed and faulty pronunciation of the Latin and Greek technical terms used in our science.

As you already know, I have chosen for my paper a subject dealing with the pathology of the spinal cord, with which I have been occupied for many years, and which has, as far as I can see, arrived, after many difficulties, at a definite scientific conclusion.

It is now exactly forty years since I ended my studies and began my practical and scientific career under the eye and guidance of Nicolaus Friedreich, who just at that time was engaged in his epoch-making observations on that disease of the spinal cord which still bears his name "hereditary ataxia" (Friedreich's disease). I was, therefore, early initiated into the pathology of the spinal cord and nervous diseases generally.

Spinal Cord Pathology.

When I look back upon those days and compare the state of our knowledge of diseases of the spinal cord at that time with our perfected understanding of the present day, I must say that probably no other branch of nerve pathology has undergone such extraordinary development and has advanced to the same degree as the pathology of the spinal cord. We are, doubtless, still far from the goal even in this branch as regards an exact understanding of its pathologico-anatomical bearings, of development and causes, and of its relations to the clinical symptoms observed; nevertheless, we may justly look with pride upon the standpoint which has been reached to-day in the pathology of the spinal cord.

A glance at the handbooks and textbooks on diseases of the nervous system of those days teaches us how incomplete our knowledge of its anatomy and pathology was, and how deficient our clinical distinction between the several forms of disease. One was just beginning to define and study more closely the anatomical foundation of locomotor ataxia, and in the seventh decade of the last century men began everywhere to study clinically and anatomically the spinal cord in particular (I need only mention the names of Duchenne, Lockart Clarke, Friedreich, Leyden, Westphal, and especially that of Charcot and his school, Vulpian and others), which led to rapid strides in broadening and deepening our knowledge. On this point I cannot go into detail here.

When I began my studies one certainly knew the diseases of the coverings of the spinal cord (meningitis, affections of the vertebrae), traumata, haemorrhages, etc., somewhat more accurately, but the diseases of the cord itself very slightly. In these two main groups were recognized to both of which the name of inflammation of the spinal cord (myelitis) was given; those forms which developed clinically with rapidity, often accompanied by fever, etc., and which showed themselves anatomically as essentially processes of softening, were classed together as acute myelitis; those forms which clinically began slowly and persisted for years, leading often to incurable disease, and which showed themselves anatomically as induration, sclerosis, grey degeneration and atrophy, were included under the term chronic myelitis.

Differentiation of "Myelitis."

It was only through elaborate methods of histological investigation and more exact clinical observation and diagnostic differentiation that a more extensive distinction and recognition of different morbid conditions was arrived at. So, taking chronic myelitis as an example, this one comprehensive term became gradually split up into the following—

* Opening Lecture Delivered at the Post-Graduate College, West London Hospital, on October 8th, 1902.